Stage-Specific Changes in Physiological and Life-History Responses to Elevated Temperature and P_{CO_2} during the Larval Development of the European Lobster *Homarus gammarus* (L.)

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ABSTRACT

An organism's physiological processes form the link between its life-history traits and the prevailing environmental conditions, especially in species with complex life cycles. Understanding how these processes respond to changing environmental conditions, thereby affecting organismal development, is critical if we are to predict the biological implications of current and future global climate change. However, much of our knowledge is derived from adults or single developmental stages. Consequently, we investigated the metabolic rate, organic content, carapace mineralization, growth, and survival across each larval stage of the European lobster *Homarus gammarus*, reared under current and predicted future ocean warming and acidification scenarios. Larvae exhibited stage-specific changes in the temperature sensitivity of their metabolic rate. Elevated P_{CO_2} increased C : N ratios and interacted with elevated temperature to affect carapace mineralization. These changes were linked to concomitant changes in survivorship and growth, from which it was concluded that bottlenecks were evident during *H. gammarus* larval development in stages I and IV, the transition phases between the embryonic and pelagic larval stages and between the larval and megalopa stages, respectively. We therefore suggest that natural changes in optimum temperature during ontogeny will be key to larvae survival in a future warmer ocean. The interactions of these natural changes with elevated temperature and P_{CO_2} significantly alter physiological condition and body size of the last larval stage before the transition from a planktonic to a benthic life style. Thus, living and growing in warm, hypercapnic waters could compromise larval lobster growth, development, and recruitment.

Keywords: Homarus gammarus, ocean warming, ocean acidification, life history, larval development, seafood.

Introduction

The persistence of marine species with complex life histories requires the successful completion of all ontogenetic stages at local and global scales (Byrne 2012). Changes in life-history traits and subsequent population dynamics of such species can be linked to the environment via physiological processes (Calow and Forbes 1998; Ricklefs and Wikelski 2002; Young et al. 2006). Two major climate-related issues are the rapid increase in global mean values for sea surface temperature and rising levels of oceanic P_{CO_2}. By 2100, ocean surface temperatures are predicted to have increased by between 3° and 5°C (Sokolov et al. 2009; IPCC 2013), while atmospheric P_{CO_2} levels are predicted to increase to 1,000 μatm from their preindustrial levels of 280 μatm, driving a reduction in oceanic pH of approximately 0.4 units (Caldeira and Wickett 2003, 2005; IPCC 2013). Coupled with decreasing pH are changes to other ocean carbonate-chemistry parameters, such as dissolved inorganic carbon and carbonate saturation states (Feely et al. 2004). Understanding the physiological responses underpinning changes to life-history traits due to complex climate change is critical if we are to link organismal and population responses to such change (Pörtner 2010). This is also important if we are to subsequently provide guidelines for stakeholders and policymakers to successfully deliver sound stock management and species conservation strategies (Wikelski and Cooke 2006; Young et al. 2006). Despite the urgent need for a good understanding of the physiological processes underlying and mediating responses of marine larvae to climate-change drivers, physiological traits are understudied compared with more conventional life-history

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traits (such as growth and survivorship), particularly in marine crustaceans (Whiteley 2011).

Temperature is a major driver of crustacean larval development (e.g., Lindley 1998; Hartnoll 2001; Daoud et al. 2010) and is largely responsible for determining development time, survival, and size at maturity (Atkinson 1995; Weiss et al. 2010). Maximum larval growth and survival occur at optimum temperatures (Sastry and McCarthy 1973), which in the American lobster *Homarus americanus* was estimated to be around 18°C, as growth and survival decreased above or below 18°C (MacKenzie 1988). The underlying physiological responses to elevated temperature in crustacean larvae are, comparatively speaking, poorly studied. Larval respiration rates increase with increasing temperature up to a maximum value, beyond which respiratory gas exchange is compromised (Schatzlein and Costlow 1978; Vernberg et al. 1981; Anger 1987). At this point larvae exhibit oxygen- and capacity-limited thermal tolerance similar to that of adults (Storch et al. 2009*a*, 2009*b*). However, both the ontogeny of aerobic scope and metabolic rates during larval development are still largely unknown. Given that the development of optimal thermal windows defines, in part, a species' biogeographic ranges (Pörtner 2001), this understanding is critical if we are to predict the effects of ocean warming on species distributions and population dynamics (e.g., Storch et al. 2009*a*, 2009*b*, 2011; Bartolini et al. 2013).

In addition to changes in temperature, elevated P_{CO_2} elicits a range of life-history responses in larval crustaceans, including increased mortality (e.g., Kurihara et al. 2004; Walther et al. 2010; Long et al. 2013), reduced survival or growth (e.g., Kurihara et al. 2008; Walther et al. 2010), and little or no effect (Arnold et al. 2009; Arnberg et al. 2013; Carter et al. 2013; Ceballos-Osuna et al. 2013). Some studies have revealed subtle, but important, effects of P_{CO_2} on larval organic content and mineralization, metabolism, and energetic trade-offs between physiological processes (e.g., Arnold et al. 2009; Carter et al. 2013; Ceballos-Osuna et al. 2013; Schiffer et al. 2013). However, with the exception of Arnold et al. (2009) and Arnberg et al. (2013), these studies mainly focus on specific larval stages and may therefore miss changes in sensitivity to elevated P_{CO_2} due to ontogenetic shifts in physiological functions between stages, which may ultimately drive the responses of later larval stages to climate change (Byrne 2012; Dupont et al. 2013).

While our understanding of the independent effects of warming and ocean acidification on the physiology of marine invertebrates' larvae is rapidly growing, their co-occurrence in the future ocean makes it imperative to study their potential impact when coupled (Pörtner and Farrell 2008; Widdicombe and Spicer 2008). This is particularly relevant because elevated P_{CO_2} has been shown to increase an organism's sensitivity to elevated temperatures via narrowing of its aerobic thermal windows (Metzger et al. 2007; Pörtner and Farrell 2008). More specifically, Walther et al. (2010, 2011) explicitly related larval sensitivities to elevated temperature and P_{CO_2} to their physiological development, showing that changes in aerobic thermal windows during larval development may cause stage-specific bottlenecks. The potential for further stage-specific bottlenecks and potential carryover effects between larval stages is still largely undetermined (cf. Dupont et al. 2013), despite the importance of such bottlenecks in determining population viability (Byrne 2012).

The aim of this study was to investigate individual-larval-stage responses, in terms of both physiological and life-history traits, to both elevated temperature and elevated P_{CO_2} throughout larval development of the European lobster *Homarus gammarus*. This species was chosen because of its complex life cycle, which includes dispersive larval stages and dramatic behavioral, anatomical, physiological, and environmental transitions between pelagic larvae and benthic juveniles (Gruffydd et al. 1975; Cobb and Wahle 1994; Cobb and Castro 2006). After hatching, *H. gammarus* undergo four larval molts, with the first three larval stages, SI–SIII, being fully pelagic. SI is an important transitional stage between benthic embryos and pelagic larvae. The onset of the fourth stage, SIV, marks the transition from pelagic larvae to semipelagic, benthic-seeking megalopa. Upon settlement, the SIV megalopa metamorphoses into the first true benthic postlarval stage, SV (Charmantier et al. 1991; Cobb and Wahle 1994). In addition to the complexity of its life cycle, *H. gammarus* is of great economic importance for many fishing communities in Europe, with an estimated 4,625 t landed during 2012 (FAO 2014). Its geographic range extends from north of the Arctic Circle and along the European and Mediterranean coasts as far south as Morocco (Cobb and Wahle 1994; Cobb and Castro 2006). Owing to its economic importance, any potential changes in local population distribution or condition due to the predicted elevated temperature and P_{CO_2} levels associated with climate change would have serious socioeconomic effects on local inshore fisheries.

In order to assess the potential effect of combined global drivers on the physiological development and survival of *H. gammarus*, larvae were reared under predicted future scenarios of both ocean warming (Sokolov et al. 2009; IPCC 2013) and acidification (Caldeira and Wickett 2003, 2005; IPCC 2013). Rates of oxygen consumption were measured as an indicator of energy demand, while organic content and carapace mineralization were measured as indices of larval condition. Growth, in terms of wet body mass, selected morphometrics, and survival were measured as indices of key life-history traits. Measurement of all of these key aspects of lobster biology at each developmental stage will enable us to see how physiological changes occurring between larval stages help define the way in which life-history traits at the subsequent stage of development will respond to environmental drivers.

Material and Methods

Animal Husbandry

Ovigerous females were caught off the coast of south Cornwall in July 2011 and kept in an aquarium (1,200 L) at the National Lobster Hatchery (Padstow, UK), with seawater sourced from the nearby Camel Estuary (Padstow; 50°32′19.67″N, 4°56′5.85″W). Stock seawater was mechanically and biologically filtered and subjected to weekly water changes (salinity = 35, T = 19°C, dissolved oxygen = 8 mg L^{-1}). Females were fed twice

a week on whole mussels ad lib. Newly hatched larvae (SI, $N = 4{,}160$) were removed within 6 h of hatching with a sieve (0.5 × 0.5-mm square mesh). They were transferred to a large rearing cone (75 L) filled with constantly aerated seawater containing *N*-chloro tosylamide (2 mg L^{-1}) to prevent bacterial infection. Larvae were treated in this water for 60 min before being transferred, again with a sieve, to the experimental system described below.

Experimental Setup

The experimental system (fig. A1) consisted of six aquaria (20 L), each containing six rearing cones (2 L). Each cone was supplied with recirculating seawater (flow rate = 10 mL min^{-1}), the water having been subjected to mechanical and biological filtering. Of the six aquaria, three were designated haphazardly to the control-temperature condition and three to the elevated-temperature condition.

Control temperature was chosen as nominal 17°C to represent the seasonal average for southwest Cornwall, and elevated temperature was designated as a nominal 21°C to represent the 4°C increase associated with ocean warming predicted for the end of this century (Sokolov et al. 2009; IPCC 2013). The temperature of the water flowing to the cones was controlled by a water chiller with heating elements (SeaChill TR10, TECO, Ravenna, Italy), and water outflow into the aquaria acted as a water bath to provide stable experimental temperatures. Each cone had a mesh (0.5 mm^2 square) on the outflow, which allowed the removal of small, broken-down food waste but the retention of larger food particles and the larvae themselves. To ensure good water quality, the system was flushed with fresh seawater (flow rate = 10 mL min^{-1}) for 8 h each day.

Within each aquarium, three of the six cones were designated control P_{CO_2} (420 μatm) and the other three designated elevated P_{CO_2} (1,100 μatm). Each cone was constantly aerated, and equilibration of sea water with the desired level of P_{CO_2} was achieved by bubbling an appropriate gas mixture into the water contained in each cone. Control P_{CO_2} was produced by bubbling untreated air into the seawater contained in three of the six cones in each aquarium. Elevated P_{CO_2} was achieved by following the method of Findlay et al. (2008), in which CO_2-enriched air was injected into the seawater in the remaining six cones in each aquarium. Levels of P_{CO_2} in the air supplied to acidified cones were measured continuously throughout the exposure period with a CO_2 gas analyzer (Li-820, Li-Cor Biosciences, Lincoln, NE).

Water chemistry was monitored daily. Measurements of pH (National Bureau of Standards scale) were obtained with a pH electrode (HI-1210B/5, Hanna Instruments, Leighton Buzzard, UK) connected to a handheld pH meter (HI-98160, Hanna Instruments) that was calibrated daily with pH buffer standards (Mettler-Toledo, Leicester, UK). Temperature was measured with a thermocouple (HH802U, Omega Engineering, Stamford, CT), and salinity was measured with a refractometer (S/Mill hand refractometer, Atago, Tokyo). Seawater samples (250 mL) were taken every 5 d, fixed with $HgCl_2$ (0.02%) to eliminate microbial activity (Riebesell et al. 2010), stored in borosilicate flasks (250 mL), and maintained in dark and dry conditions until total alkalinity (A_T) was determined with an alkalinity titrator (As-Alk2, Apollo SciTech, Bogart, GA). Carbonate-system parameters of P_{CO_2} (μatm), total carbon dioxide (TCO_2, μmol kg^{-1}), bicarbonate concentration (HCO_3^-, μmol kg^{-1}), carbonate concentration (CO_3^{2-}, μmol kg^{-1}), calcite saturation (Ω_{ca}), and aragonite saturation (Ω_{ara}) were calculated from A_T, pH, temperature, and salinity with the CO_2SYS program (Lewis and Wallace 1998), with constants provided by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990). Water-chemistry parameters for all treatments during the exposure period are presented in table 1.

Table 1: Water-chemistry parameters recorded in control and experimental seawaters over the experimental period

	17°C		21°C	
	420 μatm	1,100 μatm	420 μatm	1,100 μatm
Temperature (°C)	17.2 ± .03^A	17.2 ± .03^A	21.3 ± .03^B	21.3 ± .03^B
Salinity	33.0 ± .01^A	33.0 ± .01^A	33.1 ± .02^B	33.1 ± .02^B
pH	8.08 ± .01^A	7.73 ± .01^B	8.11 ± .01^A	7.76 ± .01^B
A_T (μEq kg^{-1})[a]	2.17 ± .04	2.18 ± .04	2.22 ± .03	2.23 ± .03
P_{CO_2} (μatm)[a]	454 ± 11^A	1,154 ± 17^B	426 ± 11^A	1,181 ± 36^B
TCO_2 (μmol kg^{-1})[a]	1,981 ± 42^A	2,121 ± 34^B	1,987 ± 20^A	2,154 ± 27^B
HCO_3^- (μmol kg^{-1})[a]	1,829 ± 40^A	2,015 ± 32^B	1,806 ± 17^A	2,039 ± 26^B
CO_3^{2-} (μmol kg^{-1})[a]	135 ± 1.6^A	65 ± 1.5^B	167 ± 4.9^C	76 ± 1.3^B
Ω_{cal}[a]	3.26 ± .04^A	1.57 ± .04^B	4.06 ± .12^C	1.87 ± .03^B
Ω_{ara}[a]	2.10 ± .03^A	1.01 ± .02^B	2.60 ± .08^C	1.25 ± .02^D

Note. Data are means ± SE for all environmental parameters measured throughout the exposure period. For pH, the National Bureau of Standards scale was used. A_T = total alkalinity. P_{CO_2} = carbon dioxide partial pressure. TCO_2 = total carbon dioxide. HCO_3^- = bicarbonate concentration. CO_3^{2-} = carbonate concentration. Ω_{cal} = calcite saturation. Ω_{ara} = aragonite saturation. Superscript capital letters indicate significant differences between treatments.

[a]Parameters calculated with the CO_2SYS program (Lewis and Wallace 1998), with constants provided by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990).

Determination of Survivorship

The number of live individuals in each cone at each intermolt period was counted, and cumulative survival was expressed as the percentage of the number of individuals introduced into the cone at day 0. This also took into account individuals removed (and not replaced) for measurements. Survival was also expressed as the percentage of individuals present in each cone during the previous stage, to capture stage-specific changes in survivorship.

Estimation of Metabolic Rate

The rate of oxygen consumption (as a proxy for metabolic rate) for one individual from each cone ($N = 9$ per treatment) at each intermolt period was measured, following closely the semiclosed-respirometry method of Spicer and Eriksson (2003). Individuals were placed in blacked-out respiration chambers (40 mL) containing filtered seawater at the same temperature and P_{CO_2} as the individual had been exposed to during the experiment. Individuals were left in open chambers to adjust and recover from handling for 30 min before oxygen measurements were made. Determination of rates of oxygen consumption was carried out over a 120-min period for SI–SIII and over a 90-min period for SIV. Preliminary trials indicated that the time individuals were left recovering in open chambers before the first measurement did not significantly affect rates of oxygen consumption, and random blanks showed negligible background changes in P_{O_2} and therefore negligible background microbial respiration. Oxygen concentration (percent) was measured at the beginning and end of the incubation period with an oxygen electrode (1302, Strathkelvin Instruments, Glasgow, UK) housed in a temperature-controlled chamber (TC50, Strathkelvin Instruments) coupled to an oxygen meter (781, Strathkelvin Instruments). Rates of oxygen consumption were calculated from the decline in oxygen concentration per unit time per body mass unit in the respirometer with oxygen solubility coefficients obtained from Green and Carritt (1967) and are expressed as μmol O_2 min^{-1} g wet $mass^{-1}$ at STP.

Determination of Growth and Morphometrics

Two individuals at each intermolt period were removed from each cone ($N = 18$ per treatment). Excess water was removed by gently blotting individuals with fine tissue paper before wet body mass (WBM, mg) was measured with a precision balance (3719MP, Sartorius, Göttingen, Germany; $d = 0.1$ mg). After weighing, all individuals were laid straight and flat on their right-hand side to be photographed with a macro-enabled digital camera (Powershot A710 IS, Canon, Reigate, UK). Photographs were analyzed with ImageJ software (Rasband WS, US National Institutes of Health, Bethesda, MD) to obtain measurements of total body length (tip of rostrum to end of uropods), carapace length (CL: rear of eye socket to rear of carapace), abdomen length (AL: front of first abdominal segment to rear of fifth abdominal segment), rostrum length (tip of rostrum to rear of eye socket), and chelae length (tip to base of cheliped propodus). Individuals were then rinsed with ultrapure water, carefully blotted dry with tissue paper, and frozen at $T = -20$°C for subsequent determination of organic content and carapace mineralization.

Determination of Organic Content

The organic content (carbon, hydrogen, and nitrogen, i.e., CHN levels) of larval *Homarus gammarus* at each intermolt stage was analyzed in one individual from each cone ($N = 9$ per treatment). Individuals were freeze-dried and weighed with a high-precision balance (AT201, Mettler-Toledo; $d = 0.01$ mg). If dry mass was <2.5 mg, the complete individual was placed in a tin cup (diameter = 2 mm, height = 5 mm) and crushed. If dry mass was >2.5 mg, the individual was ground into a uniform powder with a mortar and pestle, and a 2-mg subsample was removed and placed in a tin cup. Dried and powdered samples were analyzed with an elemental microanalyzer (EA1110 CHNS, Carlo Erba, Italy, modified by Elemental Analysis, Okehampton, UK).

Determination of Carapace Mineral Content

Carapace mineral content was determined in larvae at each intermolt stage in one individual per cone ($N = 9$ per treatment). Each carapace was carefully removed with fine forceps and meticulously cleaned of all tissue under low-power magnification (×10–50, SZXI6 binocular microscope, Olympus, Tokyo). Each individual carapace was then weighed with a high-precision balance (AT201, Mettler-Toledo; $d = 0.01$ mg) before being freeze-dried for 24 h (Modulyo freeze drier, Thermo Electron, Waltham, MA) at −50°C. The dry mass of each freeze-dried carapace was determined with a high-precision balance (AT201, Mettler-Toledo; $d = 0.01$ mg) before being digested in 2 mL nitric acid (79% concentration, trace analysis grade) in a microwave digestion unit (MarsXpress, CEM, Matthews, NC). Digests were then diluted to 10 mL with ultrapure water and analyzed for $[Ca^{2+}]$, $[Mg^{2+}]$, and $[Sr^{2+}]$ with an ICP-Optical Emission Spectrometer (Varian 725-ES, Agilent Technologies, Santa Clara, CA). Carapace mineral content is expressed as μmol mg^{-1} (carapace dry mass).

Statistical Analysis

Data were first tested for the assumption of normal distributions with a Kolmogorov-Smirnov test and then tested for homogeneity of the variances with Levene's test. When assumptions were not met, residuals were analyzed against treatment to determine how much residual variation was not due to the assigned treatment. In all cases, no significant relationship between the factors investigated and residuals was found ($P \geq 0.05$). A two-way ANCOVA with WBM as a covariant was performed to analyze the effects of temperature, P_{CO_2}, and their interactions on all measurements made at each intermolt stage. Differences between treatments were determined by estimated marginal means. WBM had no significant effect on any param-

eters measured throughout larval development ($P \geq 0.05$) and was therefore removed from subsequent analysis. The variable "tank" was included in all analyses as a random factor. There was a significant effect of tank at SI. Removing tank 1 from the analysis resulted in the effect of tank no longer being statistically significant, yet all other results remained the same. It was concluded that, while significant, tank 1 had no effect on the overall analysis. In all other cases, the variable tank was found not to be significant, and it was thus removed from analysis.

Results

Survivorship

Cumulative survival to SIV of *Homarus gammarus* larvae reared under control P_{CO_2} was 2.18% at $T = 21°C$, compared to 0.56% at $T = 17°C$, this difference in survival being significant ($F_{1,35} = 36.029$, $P < 0.001$; table 2). No significant effect of elevated P_{CO_2} on cumulative survival was detected ($P \geq 0.05$). When survival was considered as relative percent change from the previous developmental stage (fig. 1), survival of larvae from SI to SII significantly decreased at the higher temperature at both P_{CO_2} levels ($F_{1,35} = 25.911$, $P < 0.001$; fig. 1) and higher P_{CO_2}, but only at $T = 17°C$ ($F_{1,35} = 4.960$, $P = 0.033$; fig. 1). There were no further significant effects of elevated P_{CO_2} on survival from the previous developmental larval stage of *H. gammarus* ($P \geq 0.05$; fig. 1). Survival from SII to SIII and from SIII to SIV was significantly greater at the elevated temperature ($T = 21°C$; $F_{\min 1,35} = 6.107$, $P = 0.026$; fig. 1). There were no significant effects of the interactions between elevated temperature and P_{CO_2} on survival throughout larval development ($P \geq 0.05$; fig. 1).

Growth and Morphometrics

Under control conditions, WBM between SI and SIV increased from 12.6 ± 0.4 to 51.3 ± 1.5 mg, while CL and AL increased

Table 2: Selected life-history traits, survival, and growth of *Homarus gammarus* throughout larval development under elevated temperature and P_{CO_2}

	17°C		21°C	
	420 μatm	1,100 μatm	420 μatm	1,100 μatm
Cumulative survival (%):				
Stage II	72.9[A]	64.2[B]	57.5[C]	55.8[C]
Stage III	11.0	9.1	12.9	12.4
Stage IV	.6[A]	.6[A]	2.1[B]	2.2[B]
Duration (d):				
Stage I	4.0 ± .0	4.0 ± .0	3.0 ± .0	3.0 ± .0
Stage II	6.7 ± .1	6.6 ± .2	5.0 ± .0	5.0 ± .0
Stage III	13.8 ± 1.7[A]	14.2 ± 1.7[A]	8.2 ± .3[B]	7.4 ± .2[B]
Total	24.6	24.9	16.2	15.4
Wet body mass (mg):				
Stage I	12.6 ± .4	12.6 ± .3	12.8 ± .3	12.7 ± .3
Stage II	21.4 ± .4	21.9 ± .4	22.3 ± .4	22.7 ± .5
Stage III	31.6 ± 1.0	31.8 ± 1.0	31.9 ± 1.0	32.2 ± 1.2
Stage IV	51.3 ± 1.5[A]	50.4 ± 1.6[A]	44.5 ± 1.4[B]	45.6 ± 1.6[B]
Total length (mm):				
Stage I	8.05 ± .10	7.99 ± .11	7.95 ± .14	8.25 ± .15
Stage II	9.84 ± .12	9.97 ± .14	10.18 ± .10	10.43 ± .09
Stage III	11.36 ± .16	11.61 ± .20	11.30 ± .13	11.60 ± .18
Stage IV	13.31 ± .36[A]	13.20 ± .39[A]	12.15 ± .16[B]	12.42 ± .16[B]
Carapace length (mm):				
Stage I	2.86 ± .05	2.83 ± .05	2.82 ± .06	2.88 ± .06
Stage II	3.73 ± .45	3.82 ± .07	3.82 ± .03	4.04 ± .08
Stage III	4.62 ± .07	4.68 ± .09	4.73 ± .08	4.83 ± .08
Stage IV	5.41 ± .11[A]	5.23 ± .11[A]	4.80 ± .08[B]	4.90 ± .07[B]
Abdomen length (mm):				
Stage I	5.19 ± .08	5.16 ± .08	5.13 ± .09	5.37 ± .10
Stage II	6.11 ± .11	6.15 ± .10	6.37 ± .09	6.38 ± .08
Stage III	6.73 ± .11	6.92 ± .14	6.57 ± .07	6.77 ± .11
Stage IV	7.90 ± .26[A]	7.97 ± .28[A]	7.34 ± .10[B]	7.52 ± .11[B]

Note. Data are presented as means ± SE. Superscript letters represent significant differences between treatments.

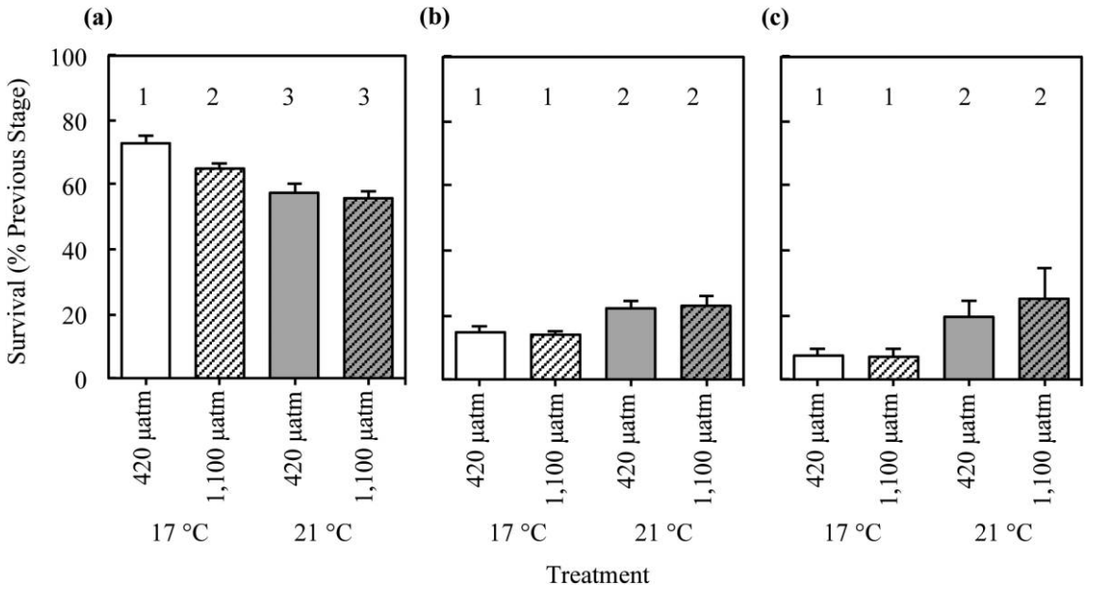

Figure 1. Survival of larval *Homarus gammarus* of each developmental stage under elevated temperature and P_{CO_2} (means ± SE); survival is expressed as percentage of live individuals from the previous developmental stage. *a*, Stage II; *b*, stage III; *c*, stage IV. White bars represent 17°C, and gray bars represent 21°C. Open bars represent 420 μatm P_{CO_2}, and hatched bars represent 1,100 μatm P_{CO_2}. Different numbers above bars highlight significant differences between treatments within stages.

from 2.86 ± 0.05 to 5.41 ± 0.11 mm and from 5.19 ± 0.08 to 7.90 ± 0.26 mm, respectively (table 2). There were no significant effects of elevated temperature or P_{CO_2}, in isolation or in consort, on WBM, CL, or AL of SI, SII, or SIII individuals ($P \geq 0.05$). At SIV, WBM, CL, and AL were all significantly higher at $T = 17$°C than at $T = 21$°C ($F_{\min 1,57} = 2.391$, $P_{\max} = 0.014$; fig. 2). There was no significant effect of elevated P_{CO_2}, in isolation or in consort with elevated temperature, on SIV WBM, CL, or AL ($P \geq 0.05$). Finally, there were no visible signs of abnormal development due to elevated temperature or P_{CO_2} noted during the exposure period.

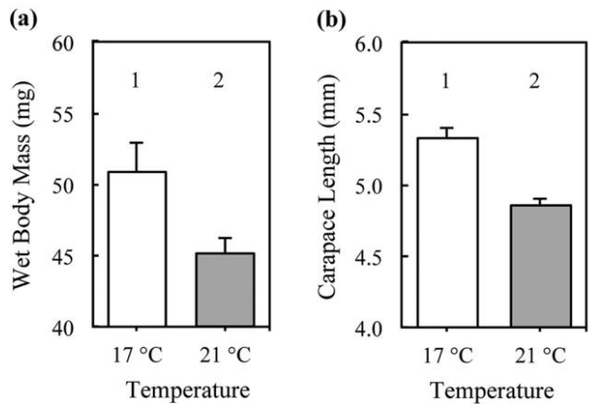

Figure 2. Effect of elevated temperature on the growth of stage IV *Homarus gammarus* larvae (means ± SE): *a*, wet body mass (mg); *b*, carapace length (mm). White bars indicate 17°C and gray bars 21°C. Control and elevated-P_{CO_2} treatments were pooled at each temperature. Different numbers above bars represent significant differences between treatments.

Rates of Oxygen Consumption

Rates of oxygen consumption under control conditions decreased from 0.42 ± 0.03 to 0.28 ± 0.02 μmol min^{-1} g^{-1} (STP) between SI and SIV. There were no significant effects of interactions between elevated temperature and P_{CO_2} on the rates of oxygen consumption at any stage of larval development ($P \geq 0.05$). There were significant effects of elevated temperature and P_{CO_2} in isolation, which we discuss stage by stage. At SI, rates of oxygen consumption were significantly lower at $T = 21$°C than at $T = 17$°C ($F_{1,31} = 9.930$, $P < 0.0001$; fig. 3), with no significant effect of elevated P_{CO_2} ($P \geq 0.05$). At SII and SIII, rates of oxygen consumption were significantly higher at $T = 21$°C than at $T = 17$°C ($F_{\min 1,31} = 25.793$, $P < 0.0001$; fig. 3). There were, again, no significant effects of elevated P_{CO_2} on the rates of oxygen consumption at SII or SIII ($P \geq 0.05$). At SIV, the rates of oxygen consumption were significantly higher at 1,100 μatm P_{CO_2} than at 420 μatm P_{CO_2} ($F_{1,30} = 4.550$, $P = 0.042$; fig. 4*d*), with no significant effect of elevated temperature ($P \geq 0.05$; fig. 3).

Organic Content

Dry mass (DM, percent of WBM) of larval *H. gammarus* reared under control conditions ranged from 17.25% ± 0.33% at SI

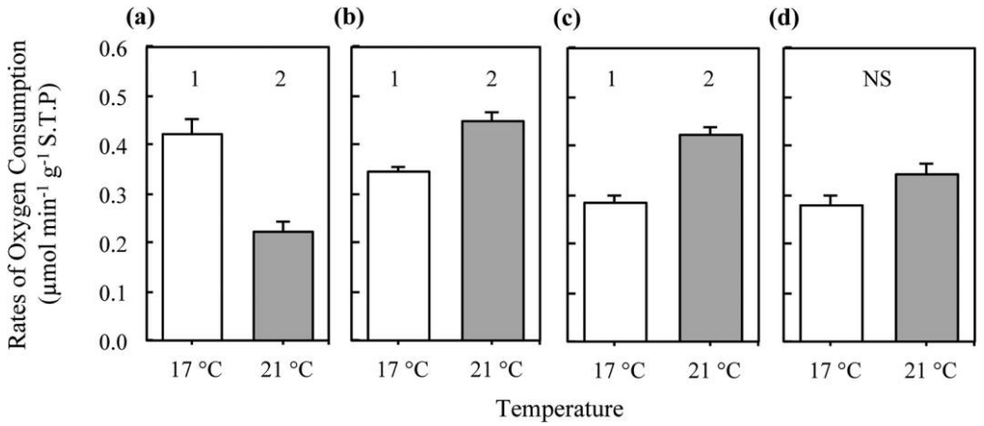

Figure 3. Effect of elevated temperature on the rates of oxygen consumption (μmol O_2 min^{-1} g^{-1}, STP) of larval *Homarus gammarus* (means ± SE) at stage I (*a*), stage II (*b*), stage III (*c*), and stage IV (*d*). White bars indicate 17°C and gray bars 21°C. Control and elevated-P_{CO_2} treatments were pooled at each temperature. Different numbers above bars represent significant differences between treatments; NS = not significant.

to 12.40% ± 3.23% at SIV, and ash content (percent of DM) ranged from 49.59% ± 0.47% to 59.58% ± 1.20%. Between SI and SIV, nitrogen content (percent of DM) ranged from 8.61% ± 0.11% to 6.65% ± 0.28%, while carbon and hydrogen content (percent of DM) ranged from 36.14% ± 0.30% to 29.38% ± 0.87% and from 5.66% ± 0.07% to 4.39% ± 0.12%, respectively. There was a significant increase in DM and a significant decrease in N, resulting in a subsequent decrease in C:N of SIV individuals due to elevated P_{CO_2} ($F_{\min 1,24} = 4.958$, $P < 0.030$; fig. 4). There were no further significant effects of elevated temperature or P_{CO_2}, in isolation or in consort, on any other aspect of organic content of SI, SII, or SIII individuals ($P \geq 0.05$).

Carapace Mineral Content

Carapace [Ca^{2+}] and [Mg^{2+}] increased from 60.49 ± 3.10 and 6.21 ± 0.19 μmol mg^{-1} in SI individuals to 152.69 ± 12.46 and 9.56 ± 0.27 μmol mg^{-1}, respectively, in SIV individuals reared under control conditions. Carapace [Mg^{2+}] increased significantly between $T = 17$°C and $T = 21$°C under 420 μatm P_{CO_2} but not under 1,100 μatm P_{CO_2}, as indicated by the presence of a significant interaction ($F_{1,19} = 9.906$, $P = .006$; fig. 5). There were no further effects of elevated temperature and P_{CO_2}, in isolation or in consort, on any other aspect of the carapace mineral content of SI, SII, SIII, or SIV larvae ($P \geq 0.05$).

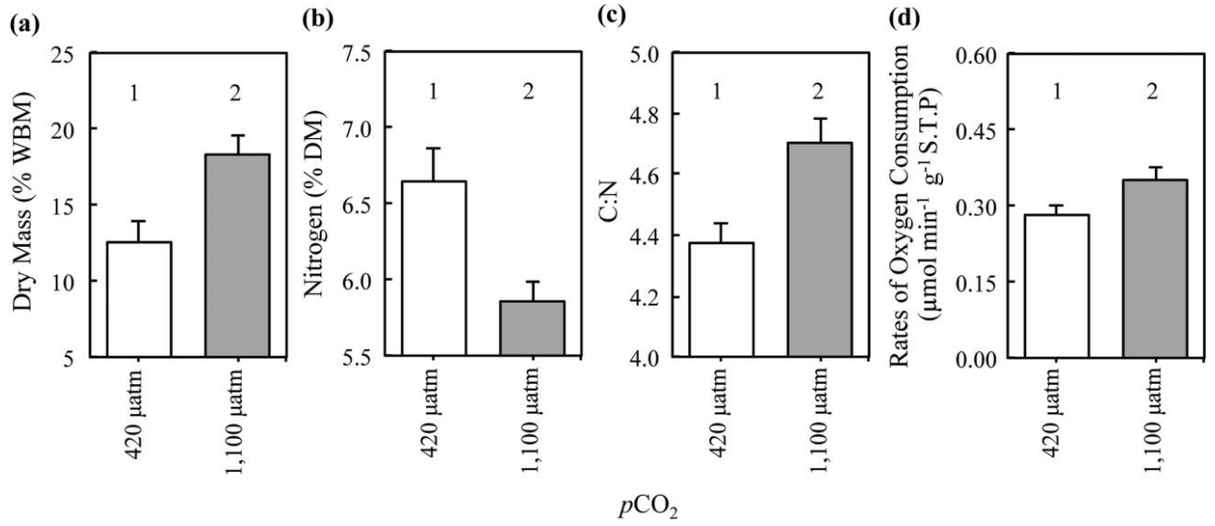

Figure 4. Effect of elevated P_{CO_2} on organic content and rates of oxygen consumption of stage IV larval *Homarus gammarus* (means ± SE): *a*, dry body mass (expressed as percent wet body mass [WBM]); *b*, nitrogen levels (expressed as percent dry mass [DM]); *c*, carbon-to-nitrogen ratio (C:N); *d*, oxygen consumption (μmol O_2 min^{-1} g^{-1}, STP). White bars indicate 420 μatm P_{CO_2} and gray bars 1,100 μatm P_{CO_2}. Control and elevated-temperature treatments were pooled at each P_{CO_2} treatment. Different numbers above bars represent significant differences between treatments.

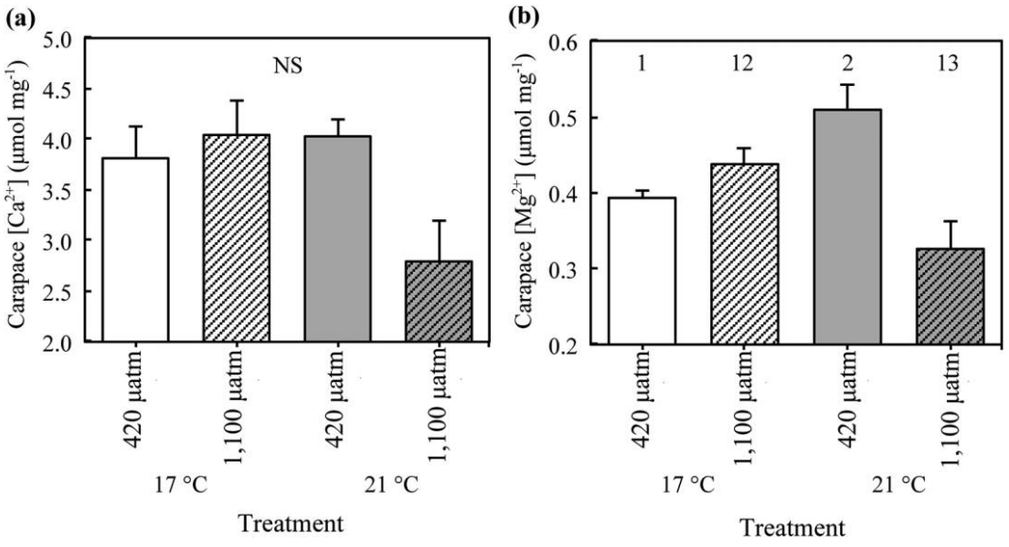

Figure 5. Effect of elevated temperature and P_{CO_2} on carapace mineralization of stage IV larval *Homarus gammarus* (means ± SE): *a*, carapace $[Ca^{2+}]$ (μmol mg^{-1}); *b*, carapace $[Mg^{2+}]$ (μmol mg^{-1}). White bars indicate 17°C and gray bars 21°C. Open bars indicate 420 μatm P_{CO_2}, and hatched bars indicate 1,100 μatm P_{CO_2}. Different numbers above bars represent significant differences between treatments; NS = not significant.

Discussion

Elevated temperature resulted in stage-specific differences in the metabolic rates of larval lobsters. SI and SIV larvae exhibited a reduction in metabolic rate at elevated temperature, perhaps suggesting energetic limitations, and lower survival, reduced growth, and greater sensitivity to elevated P_{CO_2}, compared to SII and SIII larvae. Changes in temperature sensitivity of metabolic rate between individual larval stages can inform our understanding of their life-history responses, in terms of survival and growth; our findings support the idea that life-history dynamics can ultimately be linked to the environment via physiological processes (Calow and Forbes 1998; Ricklefs and Wikelski 2002; Young et al. 2006).

Thermal Sensitivity of Lobster Larvae

Throughout the larval development of *Homarus gammarus*, elevated temperature elicited greater physiological and/or life-history responses than elevated P_{CO_2}. This was also the case for the northern shrimp *Pandalus borealis* (Arnberg et al. 2013). In *H. gammarus*, elevated temperature resulted in an increase in survival and a decrease in development time but also a decrease in body size at SIV. The overall growth and survival patterns in *H. gammarus* observed here are consistent with those reported for the larvae of other decapod crustaceans (e.g., Sastry and McCarthy 1973; Johns 1981*a*, 1981*b*; MacKenzie 1988; Anger 2001; Weiss et al. 2009*a*, 2009*b*). Survival under control conditions between SII and SIII was low, but not exceptionally so (Addison and Bannister 1994; Ennis 1995). When the underlying metabolic rates of *H. gammarus* larvae are considered, it is possible to show complex, stage-dependent responses to elevated temperature that must be carefully considered if the effects of elevated temperature on larval development are to be adequately described. Specifically, the metabolic rate of SI individuals was lower at 21°C than at 17°C, this being coupled to a subsequent decrease in survival to SII. In contrast, SII and SIII exhibited increased metabolic rates at 21°C, compared to 17°C, with higher survival to SIII and SIV. These temperature-induced changes in metabolic rate and survival between stages may represent ontogenetic shifts in optimum temperature, as reported for survival in a number of other marine crustaceans' larvae (e.g., Costlow et al. 1960, 1962, 1966). In our study, increases in survival were positively correlated with increases in metabolic rate reaction norms of the previous stage (fig. 6), indicating that in *H. gammarus* these shifts in survival may be attributed to temperature-induced changes in larvae metabolic rate. Crustaceans living above their optimal temperature range often experience a leveling off or decrease in their metabolic rates (Dehnel 1960; Sastry and McCarthy 1973; Vernberg et al. 1981; Anger 1987; Magozzi and Calosi 2015). In our study, this may indicate a decrease in SI aerobic capacity (Sastry and McCarthy 1973; Storch et al. 2009*a*, 2009*b*). Measurements of larval respiration rates integrate both active and maintenance metabolisms (Storch et al. 2009*a*, 2009*b*), and so in our study the decrease in metabolic rates suggests that at 21°C, SI larvae had reached or already passed their pejus temperatures (sensu Pörtner 2001). Subsequently, the increase in metabolic rates due to elevated temperature in SII and SIII may indicate an increased aerobic scope, compared to that of SI, and an ontogenetic shift in thermal optimum and aerobic scope during *H. gammarus* larval development, as reported in some brachyuran crabs (Sastry and McCarthy 1973).

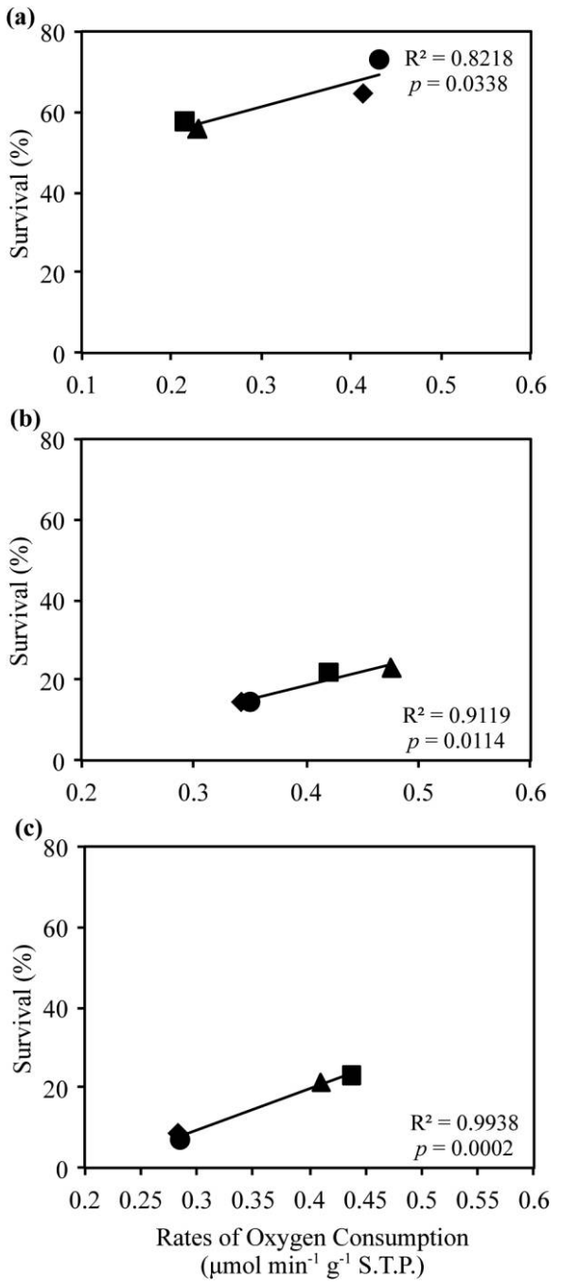

Figure 6. Relationship between the rates of oxygen consumption and subsequent survival of larval *Homarus gammarus* reared under elevated temperature and P_{CO_2}: *a*, stage I rates of oxygen consumption and subsequent survival to stage II ($y = 60.77x + 43.03$); *b*, stage II rates of oxygen consumption and subsequent survival to stage III ($y = 69.05x - 8.94$); *c*, stage III rates of oxygen consumption and subsequent survival to stage IV ($y = 102.71x - 21.36$). Circles indicate 17°C at 420 μatm P_{CO_2}, diamonds indicate 17°C at 1,100 μatm P_{CO_2}, squares indicate 21°C at 420 μatm P_{CO_2}, and triangles indicate 21°C at 1,100 μatm P_{CO_2}. Rates of oxygen consumption are expressed as μmol g^{-1} min^{-1}, STP, and survival is expressed as percent survival from previous developmental stage.

In contrast to those at other stages, SIV larvae did not exhibit a significant thermal metabolic response. This could be interpreted as a further decrease in aerobic scope (Storch et al. 2009*a*, 2009*b*) or as a developmental shift in optimal temperature conditions. This shift was accompanied by a 20% reduction in total growth between the control and elevated-temperature treatments. Temperature-related reductions in size have been attributed to decreases in development rates and increases in molt frequencies (Templeman 1936). However, in *H. gammarus* there was no significant effect of temperature on the body size of SI, SII, or SIII larvae. The decrease in body mass due to elevated temperature was confined to SIV larvae and may indicate the metabolic vulnerability of this stage to elevated temperature. This said, cumulative effects across different larval stages could not be ruled out (e.g., Dupont et al. 2013). For crustaceans, changes in WBM and body size are limited to, and defined by, the postmolt hardening phase (Anger 2001). Stage-specific temperature-related changes in growth could be attributed to energetic demands of that specific developmental stage, such as the reallocation of energy away from growth and into the development of certain physiological "milestones" (Agard 1999). Such milestones include the development of osmoregulation and respiratory regulation capabilities and structures at the transition between SIII and SIV larvae in both the Norway lobster *Nephrops norvegicus* and the American lobster *Homarus americanus* (Charmantier et al. 1988, 2001; Spicer and Eriksson 2003). It has been suggested that energy reallocation during transitional stages results in the production of smaller megalopa as a result of a mismatch between energetic demands and supply in ectotherms exposed to high temperature (Atkinson 1995).

In situ, hatching of *H. gammarus* larvae occurs in spring and early summer as seawater temperatures exceed 8°C (Richards and Wickins 1979; Charmantier and Mounet-Guillaume 1992; Cobb and Wahle 1994). Stage I larvae then rapidly concentrate at the surface (Cobb and Wahle 1994), experiencing temperatures ranging from 8°C upon hatching to 17°C in surface waters during the summer (Western Channel Observatory temperature data; D. P. Small, personal observations). Such environmental temperature shifts during larval export may be indicative of the shifting optimum temperatures between stages described in our study. Stages with narrower aerobic scopes and lower optimal temperatures (e.g., SI and SIV) are transitory stages between colder and warmer environments (SI) and vice versa (SIV). Thermal stress during these stages, therefore, will likely produce potential bottlenecks in larval development and recruitment (e.g., Bartolini et al. 2013).

Effect of Elevated P_{CO_2} on Larval Growth

While temperature appears to be the dominant driver influencing *H. gammarus* larval development, larval stages exhibiting temperature-dependent decreases in metabolic rates (SI and SIV) were also those sensitive to elevated P_{CO_2}, possibly because these stages were close to or beyond their pejus temperature thresholds (sensu Pörtner and Farrell 2008; Storch

et al. 2009*a*, 2009*b*). Early larvae and spawning adults are proposed to be the life-history stages least tolerant to elevated temperature and P_{CO_2}, as a result of ontogenetic changes in physiological tolerance during development (Pörtner and Farrell 2008; Walther et al. 2011). Expanding on this, our findings show how *H. gammarus* SI and SIV larvae have the narrowest tolerance range of the four larval stages in relation to temperature. Under elevated P_{CO_2} at the control temperature (17°C), survival from SI to SII was 10% lower than that at control P_{CO_2}, which can be explained by an increase in energetic demands associated with molting in SI larvae due to elevated P_{CO_2} (Schiffer et al. 2013). Survival effects of P_{CO_2} were not detectable at high temperature, possibly because of the high mortality in relation to elevated temperature at this stage. SIV individuals also exhibited sensitivity to elevated P_{CO_2} in terms of organic content and carapace mineralization. Metabolism and DM increased in SIV individuals because of elevated P_{CO_2} at 17° and 21°C, along with a significant decrease in N content and a consequent increase in the C : N ratio. The increase in metabolism indicates increased energy demands and, coupled with the changes in C : N ratios, suggests that increased energetic demands were accompanied by increased protein turnover, a response observed in most organisms exposed to stressful environments (Anger 2001; Weiss et al. 2009*a*, 2009*b*). Walther et al. (2010) also observed significant changes in organic content due to temperature and elevated P_{CO_2}; however, changes in C : N ratios at the megalopa stage of the spider crab *Hyas araneus* were accompanied by decreasing DM.

In terms of carapace mineralization, there was a significant effect of the interaction between elevated temperature and P_{CO_2} on SIV carapace $[Mg^{2+}]$. This resulted in carapace $[Mg^{2+}]$ significantly increasing with elevated temperature but decreasing because of elevated P_{CO_2} at elevated temperatures. SIV carapace $[Ca^{2+}]$ was not affected by elevated temperature or P_{CO_2}. Decreases in carapace mineralization have previously been demonstrated for SIV *H. gammarus* (Arnold et al. 2009) and larval *H. araneus* (Walther et al. 2011), although, interestingly, adult *H. americanus* exposed to elevated P_{CO_2} increased mineralization levels (Ries et al. 2009). The decrease in carapace $[Mg^{2+}]$ due to elevated P_{CO_2} at 21°C, compared to an increase due to elevated P_{CO_2} and temperature at 17°C, supports the idea of an elevated temperature-related increase in sensitivity to elevated P_{CO_2}. The responses in terms of organic content and mineralization in this study, as well as those observed by Arnold et al. (2009) and Walther et al. (2011), indicate changes in resource allocation, possibly because of the development of physiological structures and functions at megalopa stages (Agard 1999) and possibly because of the energetic demands generally during larval development (Arnold et al. 2009; Walther et al. 2010, 2011). Altogether, the current observations provide a mechanistic basis for the effect of elevated P_{CO_2} on developmental stages and corroborate the idea that particular life stages will represent critical bottlenecks for population growth and stock sustainability during times of ocean change (e.g., Walther et al. 2010; Byrne 2012).

Conclusions

Understanding the physiological ontogeny of marine larvae in the light of ocean change is crucial if we are to understand populations' potential responses to ocean change. Alterations to larval body size, organic content, and carapace mineralization at the pelagic-benthic transition phase (i.e., megalopa stage) due to elevated temperature and P_{CO_2} may have profound ecological consequences affecting the survival, growth, and performance of subsequent juvenile stages (Jarrett and Pechenik 1997; Pechenik et al. 2002; Jarrett 2003; Giménez et al. 2004; Nasrolahi et al. 2012; Pansch et al. 2012). Equally, the transition between embryonic and SI larvae, the benthic-pelagic transition phase, may also be of great importance for modulating population dynamic processes, because physiological sensitivity in early developmental stages has been linked to the occurrence of extreme climatic events and the reduction in the number of adults (Bartolini et al. 2013). While *H. gammarus* recruitment in terms of overall survival may appear to increase with elevated temperature, the associated temperature-related decrease in megalopa body size could also have important negative consequences, as demographic processes of lobster stocks are body size dependent (Wahle 1992; Wahle and Steneck 1992; Cobb and Wahle 1994; Wahle and Fogarty 2006), with larger size at settlement leading to greater survival (Wilbur 1980). If we consider that, under elevated-P_{CO_2} conditions, SIV individuals appear to undergo energetic trade-offs between growth and condition, further negative effects of climate change on lobster recruitment and population dynamics may be expected. The current findings highlight that stage-specific sensitivity to elevated temperature and P_{CO_2} in species with complex life cycles, such as *H. gammarus*, may influence future recruitment patterns, in ways more complex than previously concluded from studies based on temperature and P_{CO_2} alone.

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APPENDIX

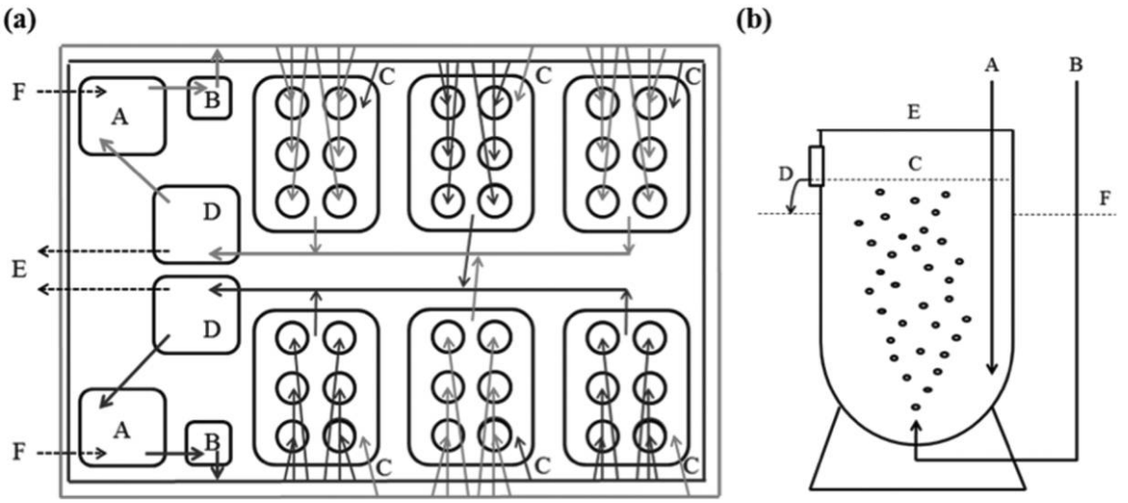

Figure A1. Diagram of the experimental setup. *a*, Temperature control; dark gray lines and arrows indicate the flow of control (17°C) treatment water; light gray lines and arrows indicate the flow of elevated-temperature (21°C) treatment water. A = header tanks with filtration. B = chiller units with heating elements to control water temperature. C = water inflow into individual rearing cones; note that there is a "swapping" of treatment water to aid temperature control within tanks. D = sump tanks with filtration. E = waste water overflow. F = daily 8-h flow of new, clean seawater to ensure water quality. *b*, Individual larval rearing cone. A = water inflow (set at designated temperature level). B = air inflow (set at designated P_{CO_2} level). C = water height in cone. D = water overflow through mesh to allow broken food removal but retain larvae and larger food particles. E = cone lid to prevent excess evaporation and spray. F = water level in surrounding tank. A color version of this figure is available online.

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